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RESEARCH ON DETECTION OF EXTRATERRESTRIAL LIFE BY ULTRAVIOLET SPECTROPHOTOMETRY

UNPUBLISHED PRELIMINARY DATA

THIRD QUARTERLY PROGRESS REPORT,

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INTRODUCTION

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The work described in this report is part of a program to study the feasibility of various methods for the detection of extraterrestrial life. The method under study in this part of the program is based on the specific absorption of a narrow region of the far ultraviolet by materials of biological origin. Absorption of ultraviolet in the 2600-2650 A region by nucleic acids and at 2800 A by many proteins is well known. The absorption of far ultraviolet in the 1850-1900 A region by peptides is being studied in this project.

The two previous reports were concerned with instrumentation problems and with the absorption of far ultraviolet by a variety of proteins, dipeptides, tripeptides and polypeptides. Evidence was presented supporting the hypothesis that the peptide bond was responsible for the observed absorption, and some preliminary experiments with soil extracts were reported.

The work described in the present report is an extension of the studies on the specificity of absorption and on the application of the principle to extracts of fertile and infertile soils.

EXPERIMENTAL

Instrumental

All absorption measurements were made with a Beckman DK-2A recording spectrophotometer modified for far ultraviolet. The studies done in the region below 2200 A were under conditions of continuous nitrogen purging.

Fused silica cells, transparent in the region of interest, were used for all measurements. Short path lengths were obtained by means of spacers inserted in a 1.0 cm cell in some cases, and by use of a Beckman short path cell in other cases.

Materials

The following materials were studied spectrophotometrically:

Tryptophane

Phenylalanine

Tyrosine

Alanine

Glycine

Serine

Leucine

Alanyl alanine

Alanyl leucine

Acetic acid

Gelatin

Bovine serums albumin

Local soil

Potomac River sand

Pepsin (as used for hydrolysis of protein)

Methods

Solutions were prepared in water and the pH was subsequently adjusted by adding hydrochloric acid or sodium hydroxide. In all the experiments, the solvent in the reference cell was the same as that used in the sample cell.

For studies on the relationship of absorbancy at 1850 A to the number of peptide bonds, bovine serum albumin and gelatin were subjected to enzymatic hydrolysis; in addition gelatin was also subjected to acid hydrolysis. The enzymatic hydrolysis were carried out by preparing a $2.4X10^{-5}$ M stock solution of the protein and a $2.4X10^{-6}$ M stock solution of the enzyme. A series of test tubes were prepared by mixing 5cc aliquots of the protein and enzyme stock solutions, and the contents of each tube were examined spectrophotometrically at periodic intervals. The reference cells in these experiments contained enzyme as well as solvent.

In studying the acid hydrolysis of gelatin, a stock solution of 1 gm gelatin in 100 cc water was prepared, and was then divided into 10 cc aliquots per test tube. A 1.0cc aliquot of 1.0 N Hcl was added to each tube, and the tubes were then placed in a bath of boiling water. Tubes were withdrawn at 30 second intervals for spectrophotometric studies.

In the case of the extracts of soil and sand, aliquots were stirred with solvent (water, sodium hydroxide or hydrochloric acid) for 20 minutes. The supernatant was filtered through a No. 42 filter and then through a millipore filter. Hydrochloric acid was added to the clear filtrate to adjust the pH, and the spectrophotometric absorption was then studied.

RESULTS

Amino Acids

Generally, the absorption of far ultraviolet in the 1850-1900 A region by aliphatic amino acids tends to disappear as the pH of the solution is lowered to a value well below the pk of the amino acid. This may be seen from table 1 which shows absorbancies for alanine, glycine, leucine, and serine. The decrease in absorbancy as the pH is lowered suggests that the carboxyl ion is involved. This suggestion led to the examination of acetic acid as representative of a simple monocarboxylic acid. The absorption spectrum of acetic acid in this region (figure 1) clearly shows an absorption band at 1830 A similar to that shown by the amino acids at higher pH values and indicates that the carboxyl ion is involved in the absorption.

The aromatic amino acids absorb in the 1850-1900 A region even at low pH values. This is seen in table 2 for tryptophane, tyrosine, and phenylalanine. Figures 2, 3, and 4 show the absorption spectra for these amino acids.

Peptides

Experiments performed with alanyl alanine and alanyl leucine as representative dipeptides confirmed previously observed absorption.

TABLE 1

ABSORBANCY OF 185-190 MW

Amino Acids	pH1	pH3	pH5	pH7	pH9	pH11
Alanine	.0000	.0414	.1875	.11021	.1072	.0645
Leucine	.0000	.1189	.1139	.0899	.0414	.1189
Glycine	.0000	.0000				
Serine	.0220	.0607	.1287		.2148	

TABLE 2

ABSORBANCY OF 185-190 MW

Amino Acids	pH1	pH3	pH5	pH7	pH9	pH11
Tyrosine	1.5224	.1522$_4$	1.2219	1.3979	1.2219	1.3979
Phenylalanine	1.3050	1.6990	1.6990	1.6990	1.6990	1.6990
Tryptophane*	.6571	.6571	.6021	.5224	.6776	.6776

* Broad band absorption

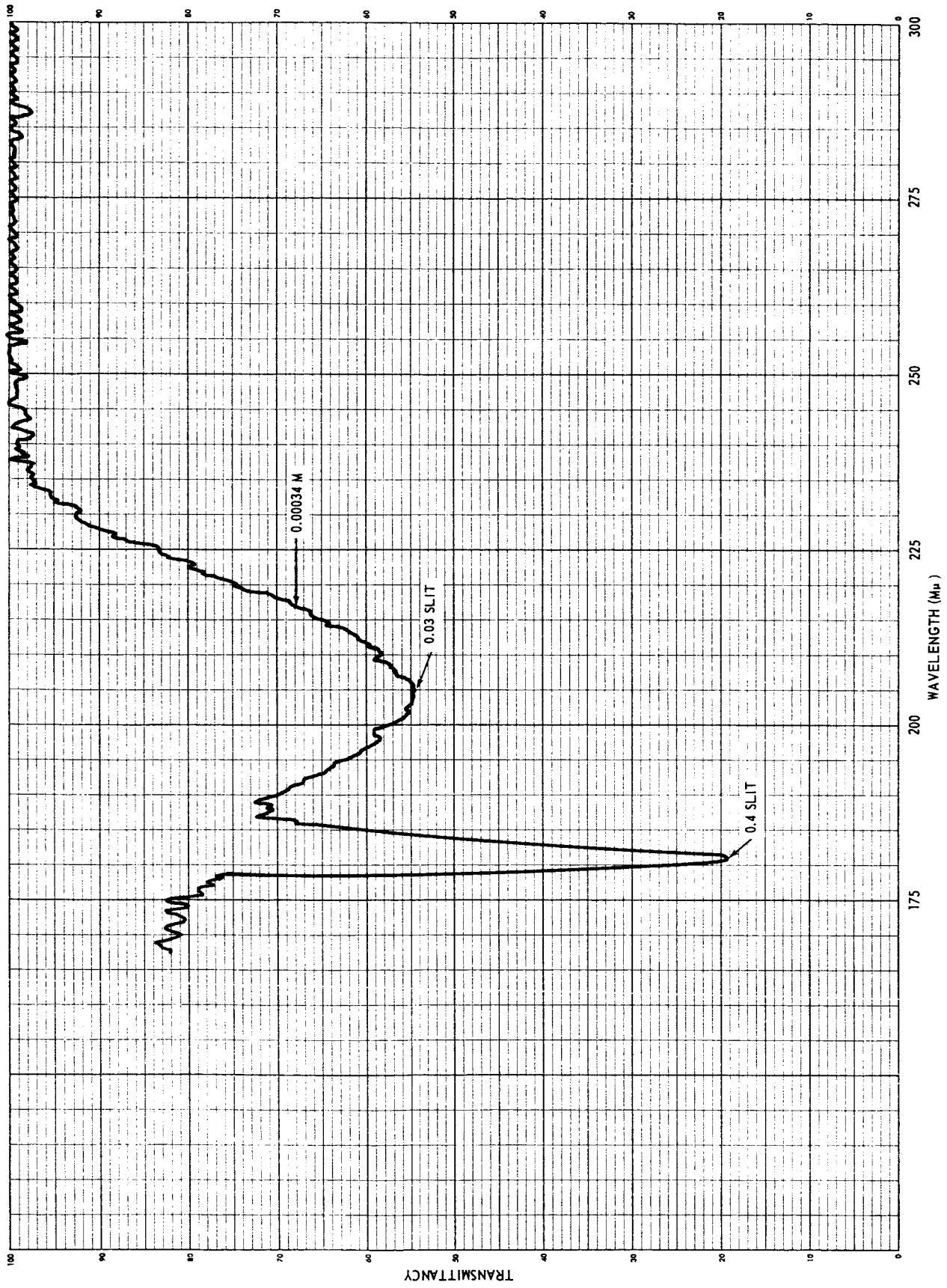

Figure 1. Absorption Spectrum - Acetic Acid

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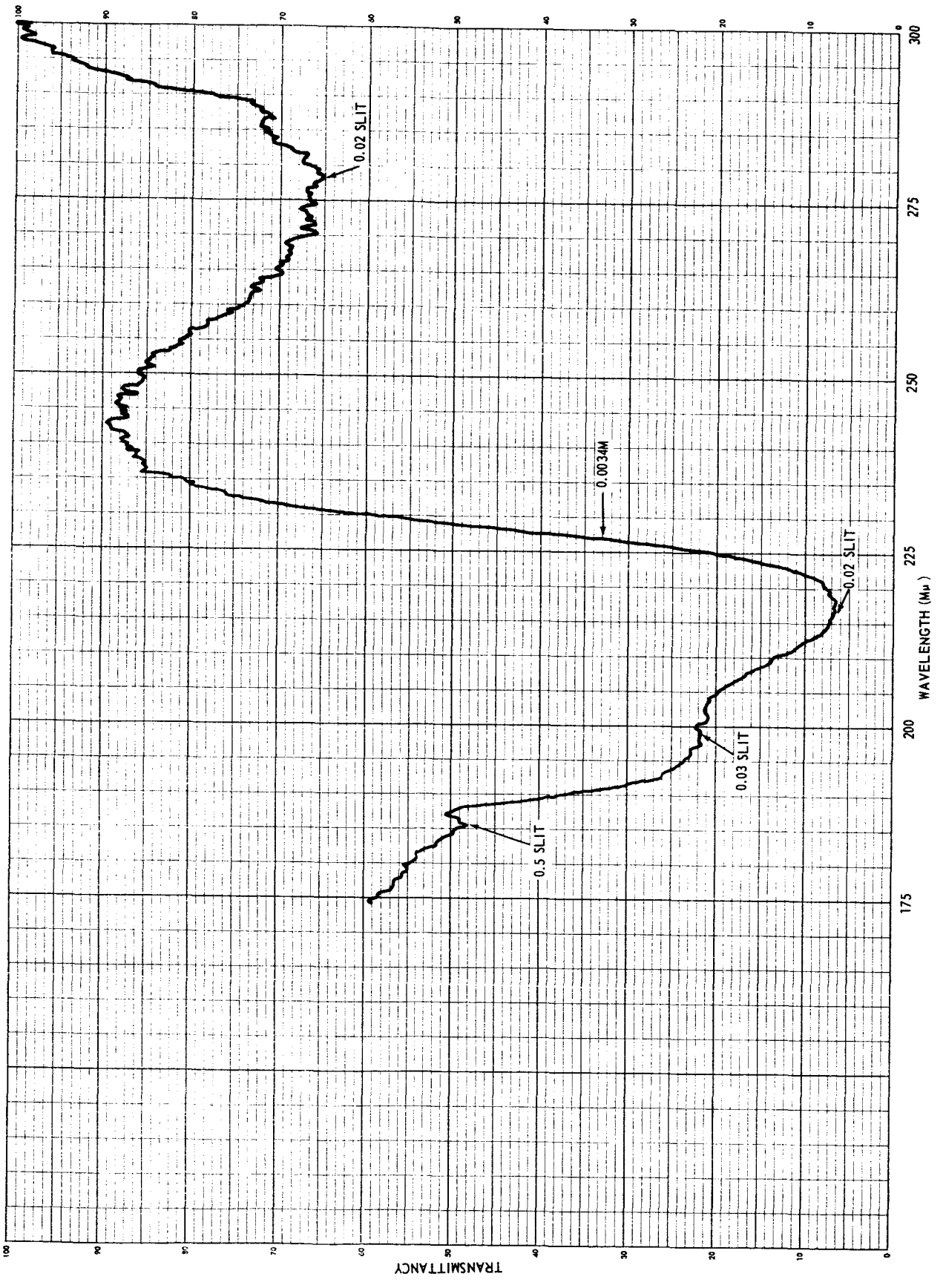

Figure 2. Absorption Spectrum - Tryptophane

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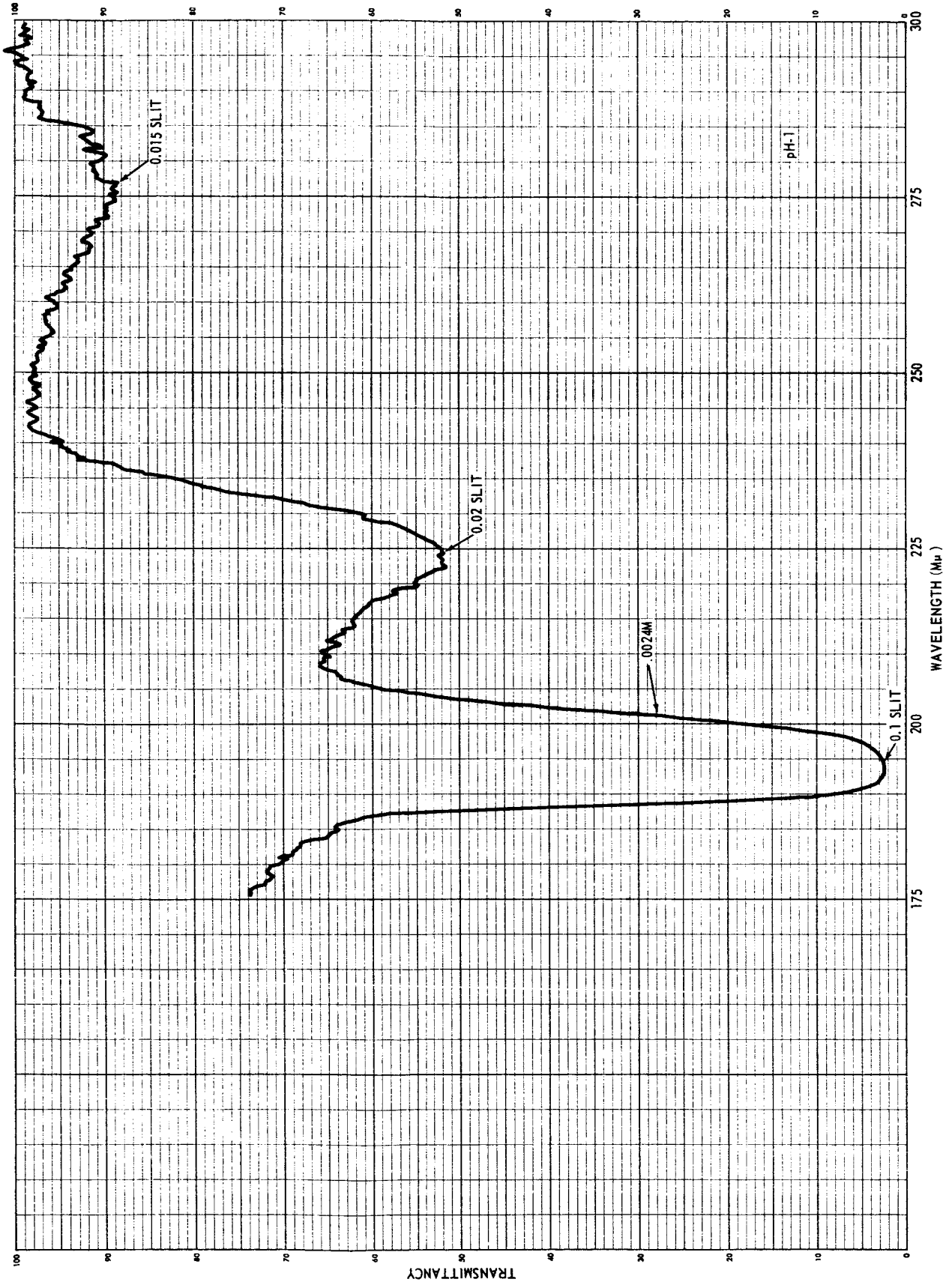

Figure 3. Absorption Spectrum - Tyrosims

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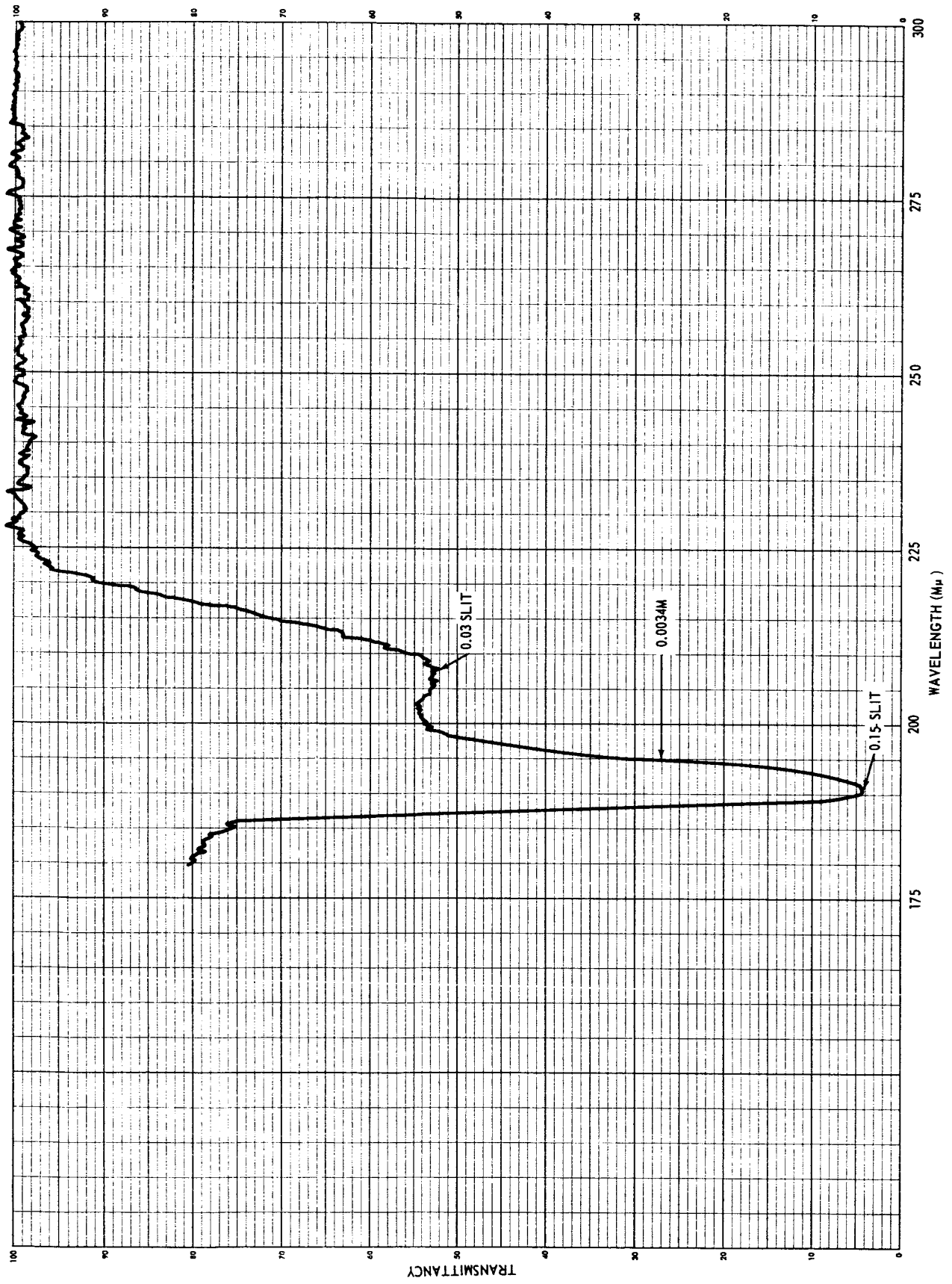

Figure 4. Absorption Spectrum - Phenylalanine

The spectra for these substances were measured against solvent in the reference cells and against equimolar quantities of alanine. The spectra obtained showed that the amino acid reference did not affect the absorption at 1850-1900 A.

Protein Hydrolysis

To further study the relationship of absorbance to the number of peptide bonds, bovine serum albumin and gelatin were hydrolyzed and the absorbancies were studied as a function of time of hydrolysis. The acid hydrolysis of gelatin went to completion as will be seen from figure 5 where absorbancy is plotted as a function of time of hydrolysis.

The enzymatic hydrolysis of gelatin proceeded as far as pepsin will carry it as is shown in figure 6. Similar results were obtained with bovine serum albumin as shown in figure 7.

Soil and sand

Extracts of local soil showed definite absorption peaks in the 185-190 Mμ region as shown in figure 8. However distinct spectral shifts were observed with changes in the extracting solvent. It will be noted that the absorption peak in the water extract is at 190 Mμ. The alkaline extract shows an absorption maximum at about 200 Mμ, while the acid extract peaks at 207 Mμ. Experiments with varying amounts of soil indicated that a significant seeding could be obtained with as little as 0.05 gms soil in 3 ml of sodium hydroxide or hydrochloric acid. Smaller amounts would be detected with water extraction.

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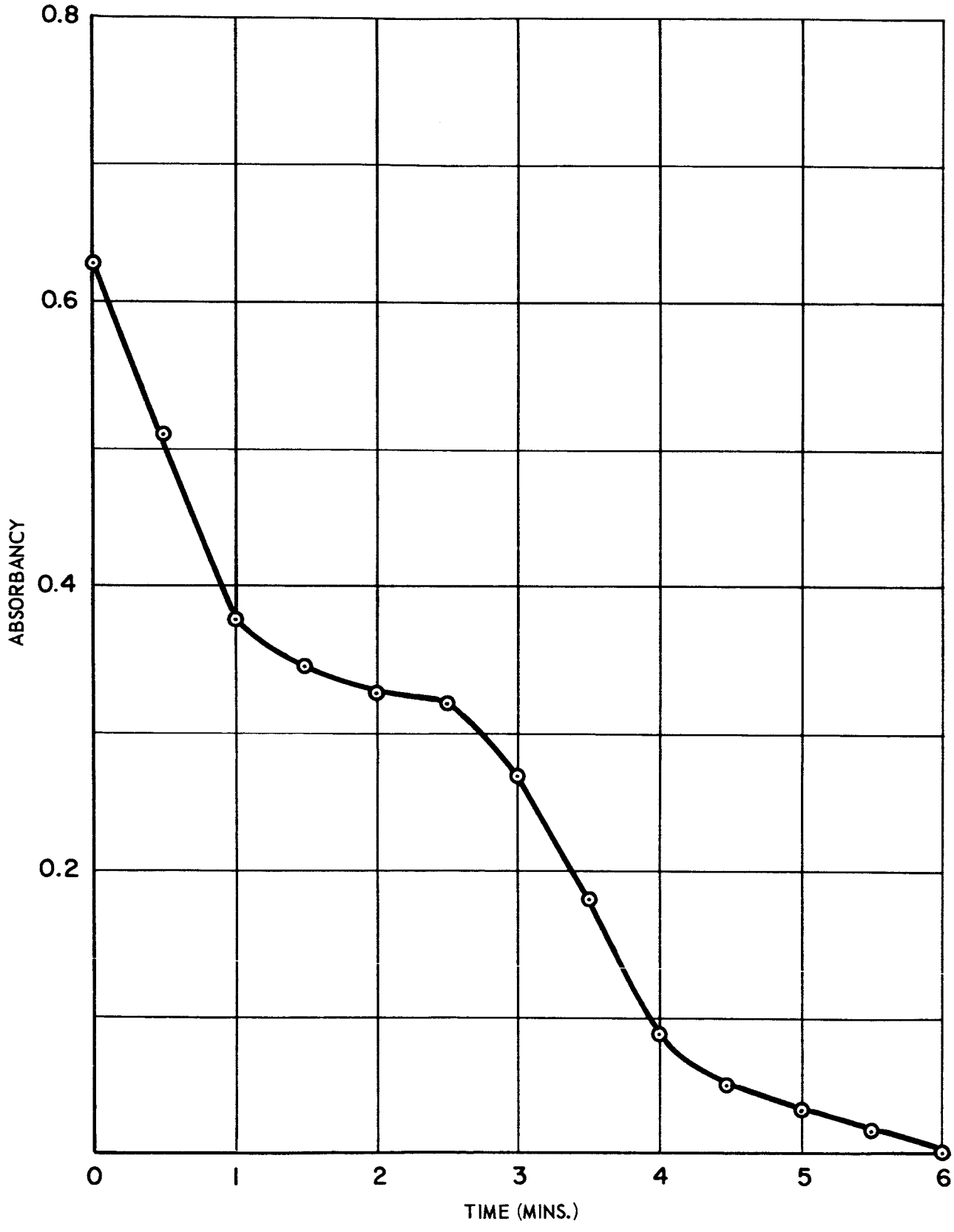

Figure 5. Acid Hydrolysis of Gelatin (Absorbancy vs. Time)

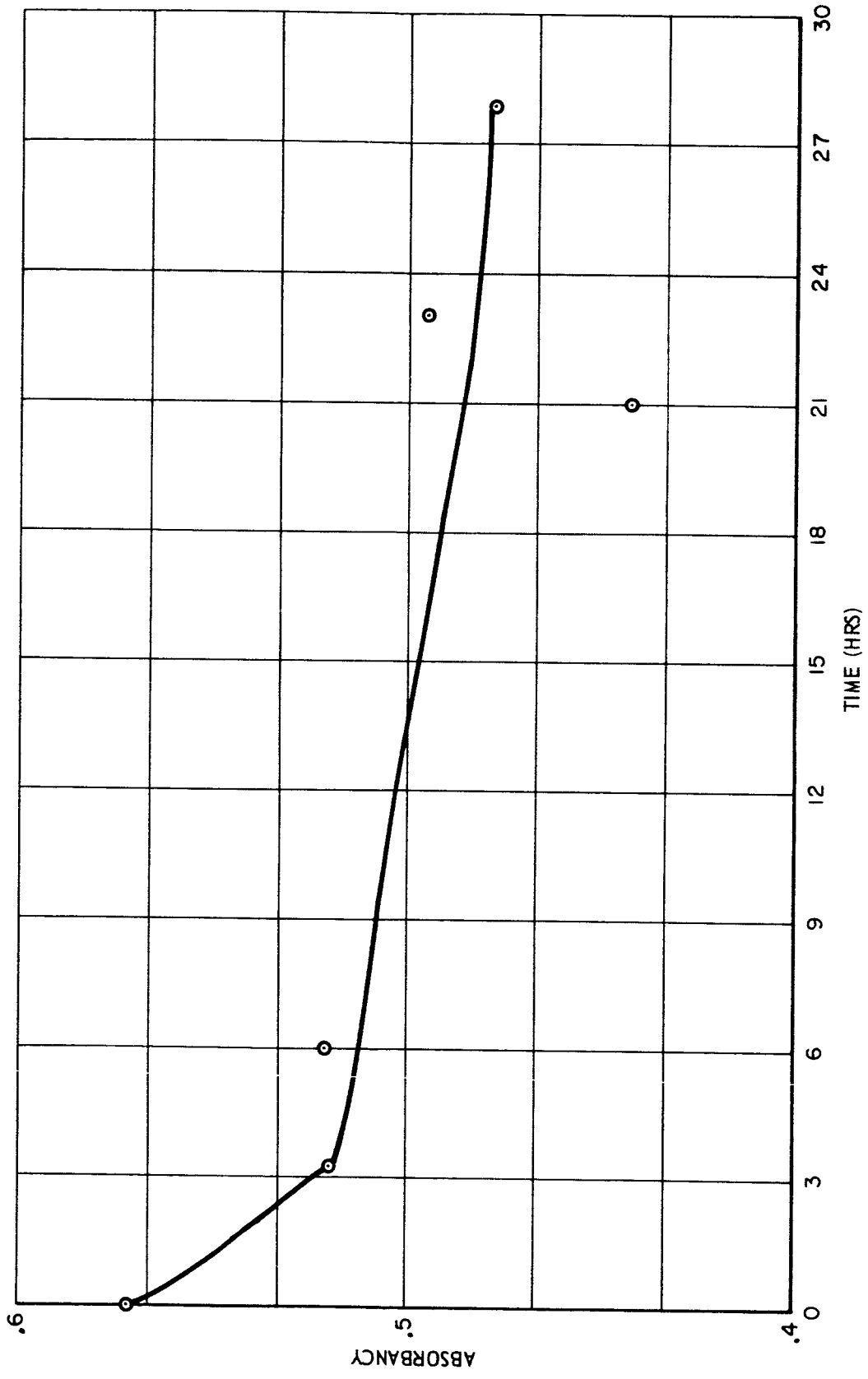

Figure 6. Pepsin Hydrolysis of Gelatin (Absorbancy vs. Time)

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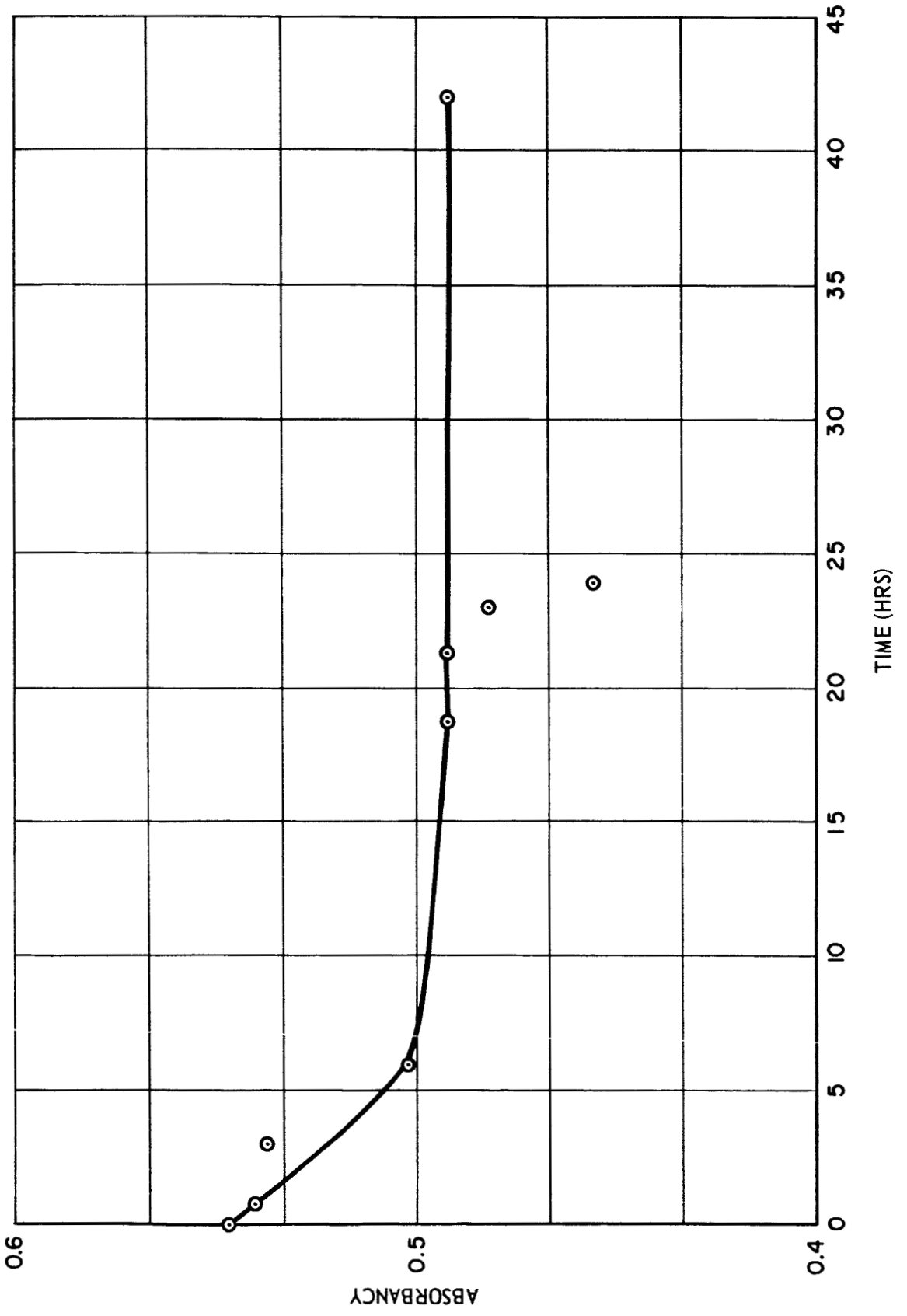

Figure 7. Pepsin Hydrolysis of Bovine Serum Albium (Absorbancy vs. Time)

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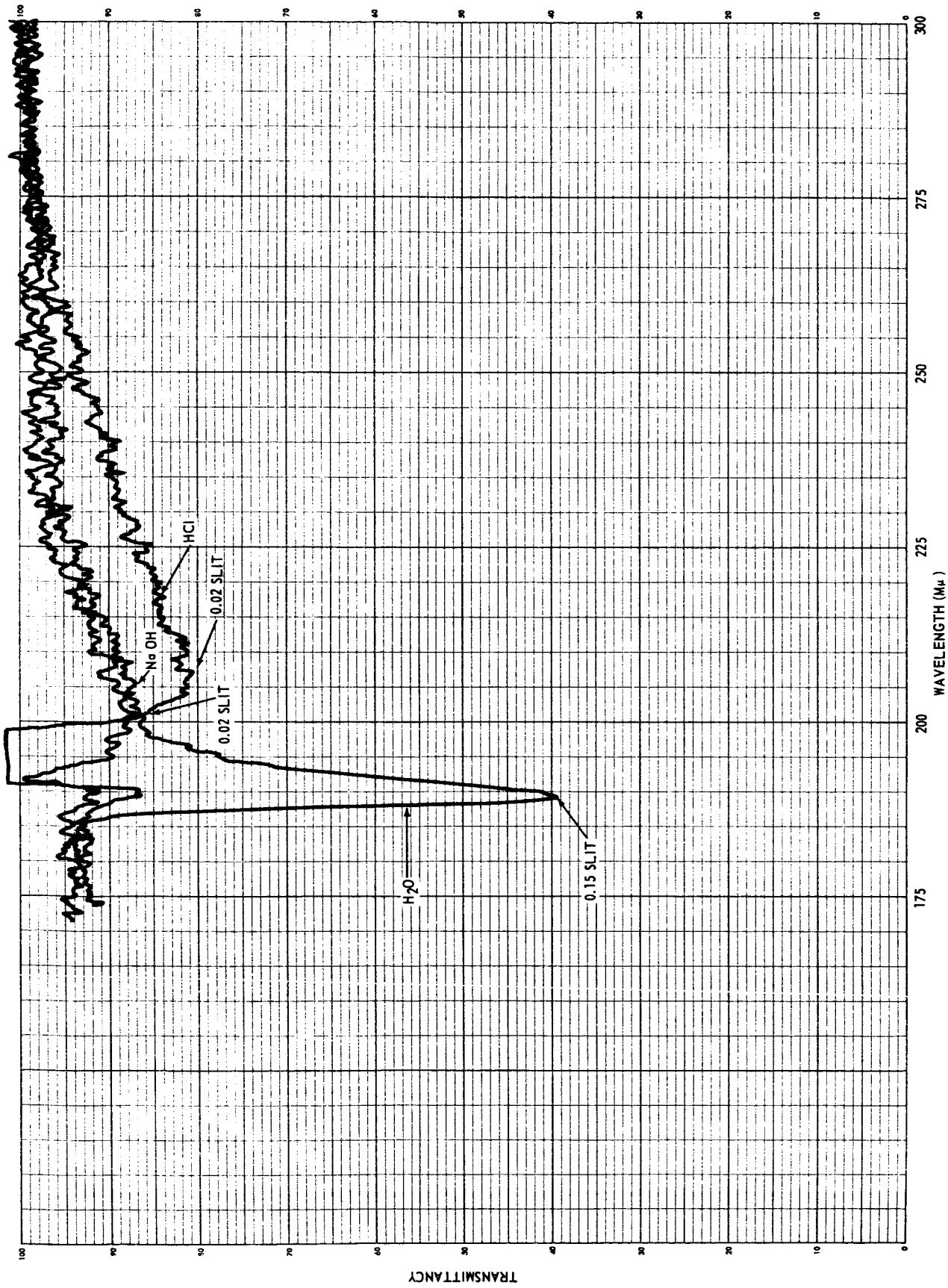

Figure 8. Absorption Spectrum - Extracts of Local Soil

Studies done with Potomac River sand indicated that significant readings could be obtained with 0.1 gm in 3 ml of extracting solvent. The absorption spectrum shown in figure 9 shows the same general characteristics as that for soil extracts, but the shift towards longer wave lengths seemed more pronounced with the acid extracts.

DISCUSSION

The results obtained during this reporting period are strongly indicative of a characteristic absorption of far ultraviolet by the peptide bond, and offer strong confirmation of the interpretation of results in previous reports.

Experiments with dipeptides, polypeptides, and proteins all confirm the presence of the absorption maximum. The hydrolysis of gelatin and bovine serum albumin is closely followed by a decrease in absorption in accordance with the working hypothesis on which the project is based. Acid hydrolysis results in the breaking of all the peptide bonds, and thus in the decrease of absorbancy to zero. On the other hand, it is well known that pepsin is capable of only about 20 percent hydrolysis and it may therefore be expected that the absorbancy will level off at some intermediate value. Any rate difference obtained in the pepsin hydrolysis reported here, as compared with literature reports, may be due to the fact that our experiments were carried out at room temperature.

The fact that the observed absorption is characteristic of the peptide bond has a great deal of significance in considering its application.

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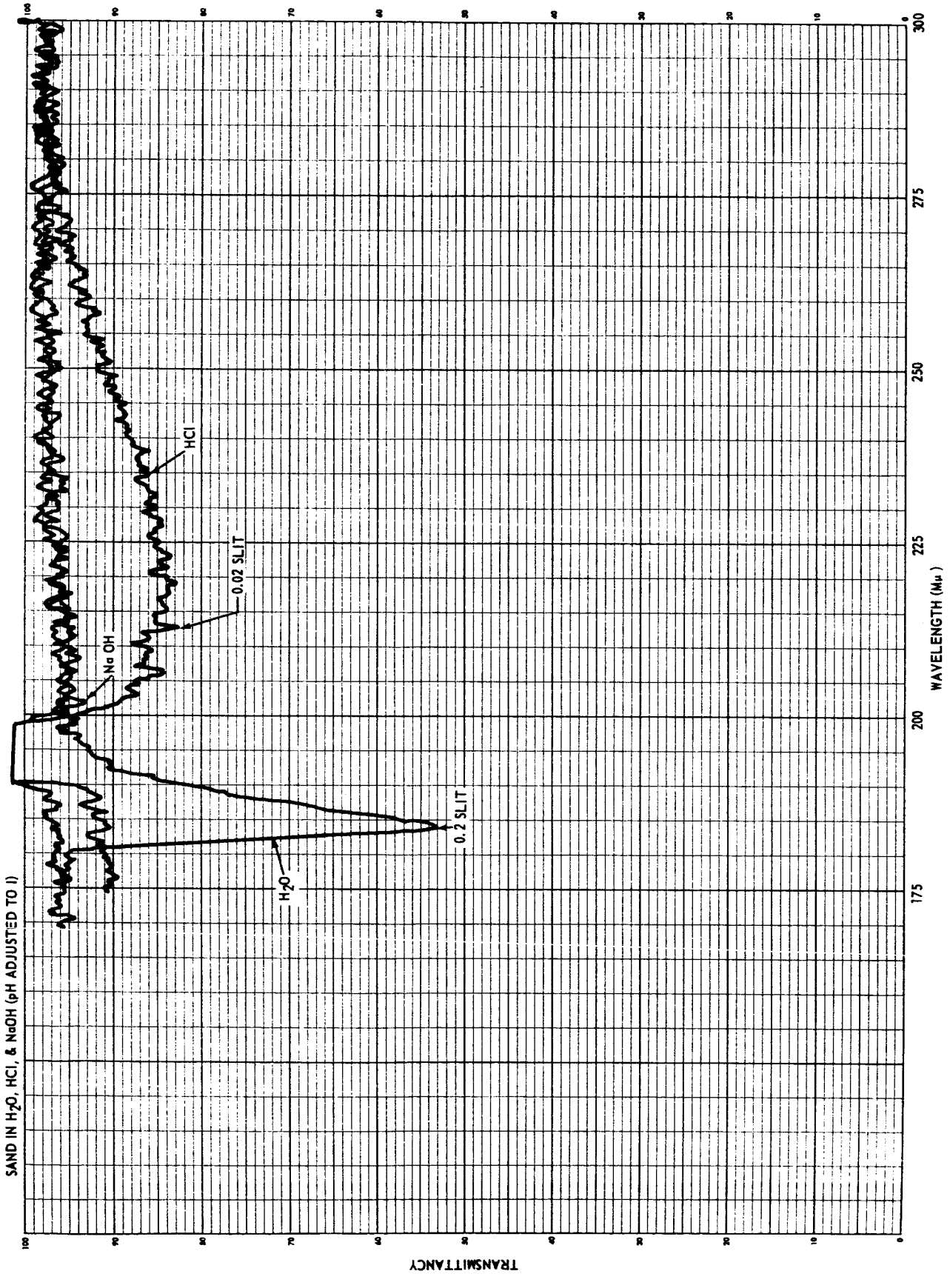

Figure 9. Absorption Spectrum - Potomac River Sand

Barring false positive responses due to interferences discussed below, the phenomenon can be applied to the detection of peptide bonds and can be extended to an approximation of the molecular weight of the material. Thus if an average molecular weight is assumed for amino acids, the molecular weight may be estimated from the absorbancy, weight concentration and the molar absorptivity.

The studies on extracts of soil and sand indicate the feasibility of applying the measurement of far ultraviolet absorption to the remote detection of extraterrestrial life. The presence of a peptide bond constitutes reasonable evidence of the presence of biological systems either past or present. The positive results obtained with soil and sand extracts are due to the presence of biological matter in these materials. The soil samples seemed quite fertile, and the detection of biological material in these could be easily anticipated. However, the samples of sand that were used lacked all outward signs of fertility; yet the detection of peptides was accomplished on a relatively small sample.

The effects of pH indicate quite clearly that the carboxyl ion absorbs in the same region as the peptide bond. This is shown by the disappearance of the absorption peak in alphatic amino acids as the pH is lowered, and in the pronounced peak exhibited by acetic acid. Knowledge of this effect provides a useful tool in the elimination of confusing absorption by substances other than peptides.

The absorption by the aromatic amino acids may be considered to be an interference, but the presence of amino acids also is indicative of living systems.

SUMMARY

Spectrophotometric measurements in the 1850-2500 A region were carried out on a series of amino acids, peptides, proteins and extracts of soil and sand. Evidence was presented indicating that the peptide bond was responsible for an intense absorption in the 1850-1900 A region. Since the carboxyl ion also absorbs in this region, it is necessary to reduce the pH to well below the pk to observe the peptide bond absorption.

Hydrolysis of gelatin and bovine serum albumin was accomplished by a decrease in absorbancy as the hydrolysis proceeded.

Present work is centered around the elimination of interferences, and the study of whole organisms such as bacteria and fungi, and the study of aerosolized background.

The specific tasks to be completed during the next quarter are:

1. Extension of work with soil to include, (a) soils from different areas, (b) soils of varying degrees of fertility, (c) soils of varying physical structure, and (d) dust.

2. Study of possible interferences in order to firmly select a reference channel to exclude interferences.

3. Studies on biological organisms such as bacteria, molds, and similar forms.

4. Preliminary investigations of factors affecting breadboard design.

5. Study of the feasibility of integrating this approach with the optical rotatory dispersion approach into a single device.